Figure 1

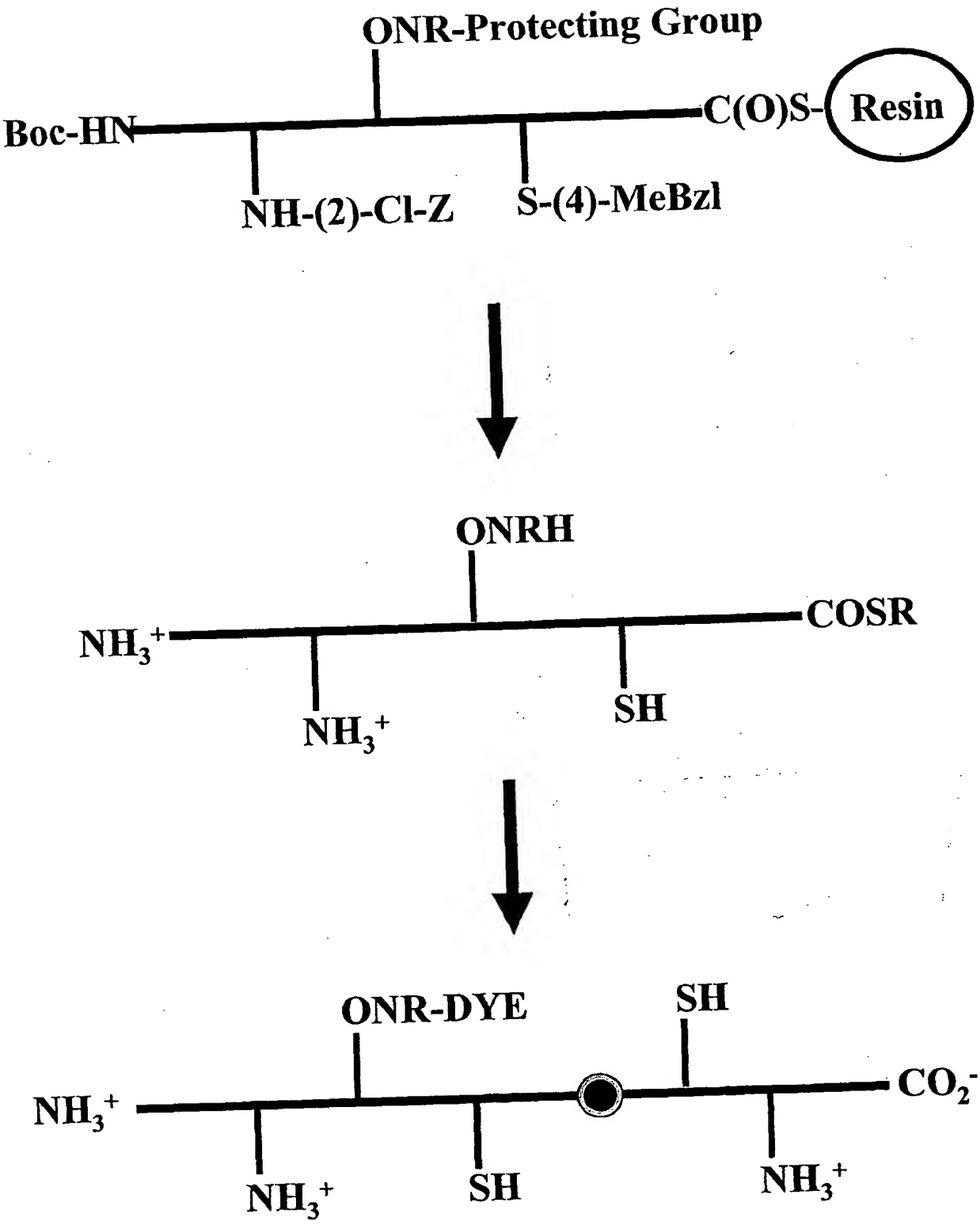
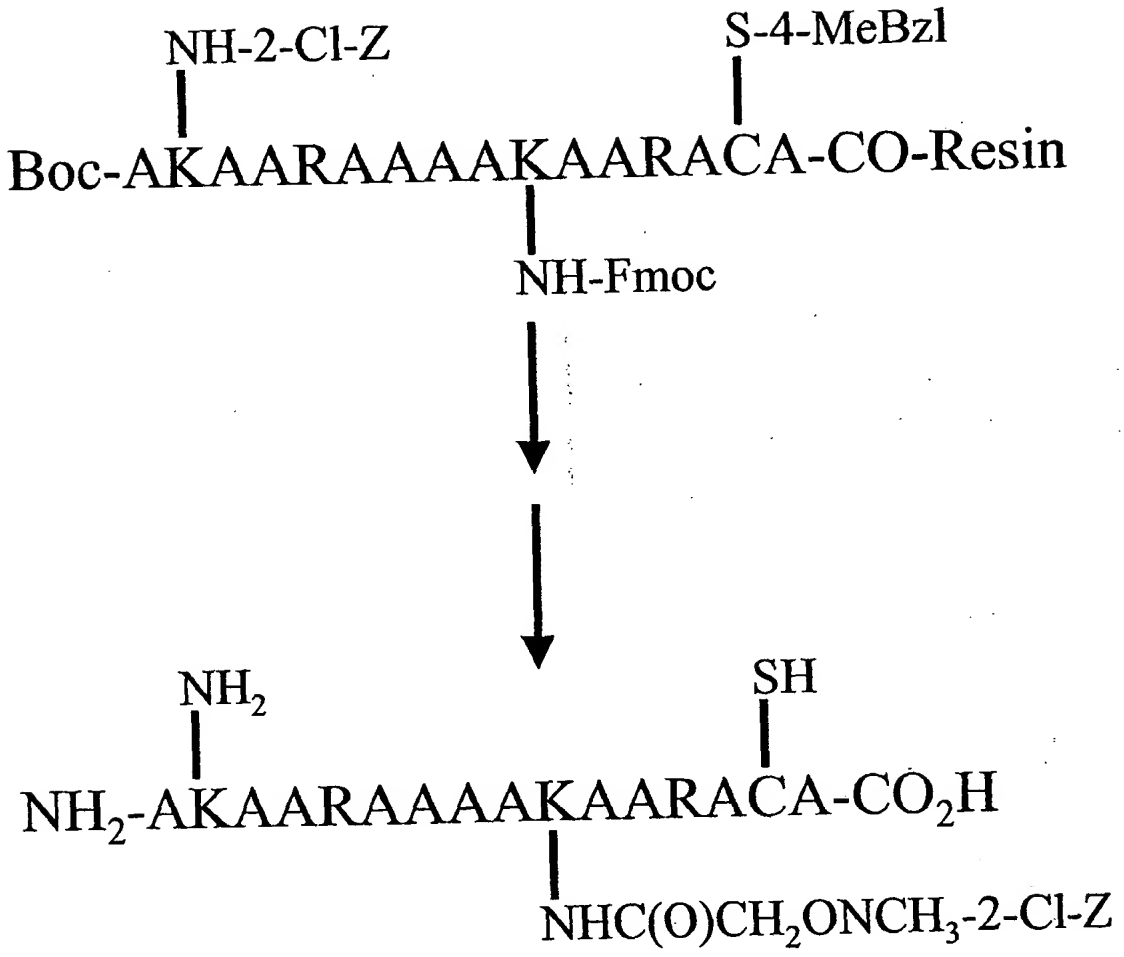

ONR-Protecting Group
Boc-HN
C(O)S-
Resin
NH-(2)-Cl-Z
S-(4)-MeBzl
ONRH
NH_3^+
COSR
NH_3^+
SH
ONR-DYE
SH
NH_3^+
CO_2^-
NH_3^+
SH
NH_3^+

Figure 2



SA-Test Peptide

Figure 3

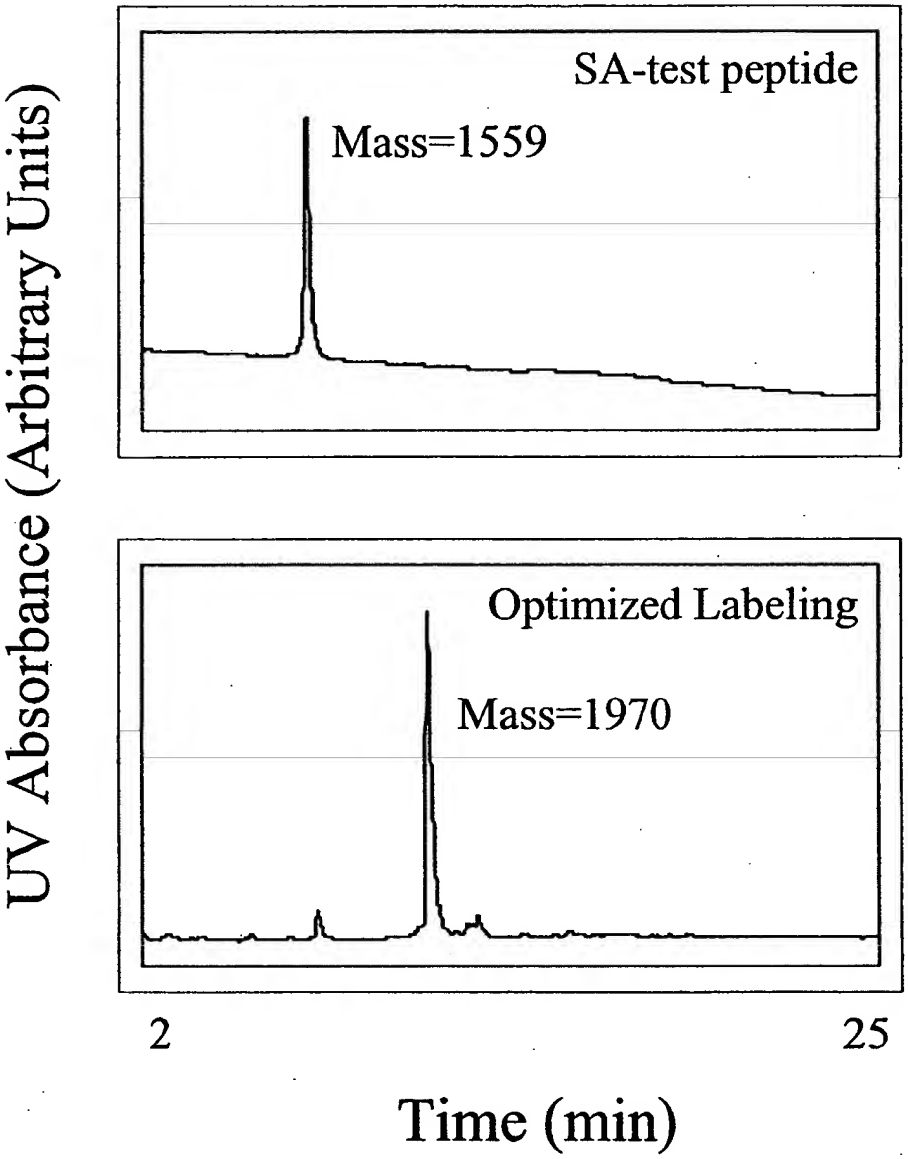

SA-test peptide
Mass=1559
Optimized Labeling
Mass=1970
UV Absorbance (Arbitrary Units)
2
25
Time (min)

Figure 4

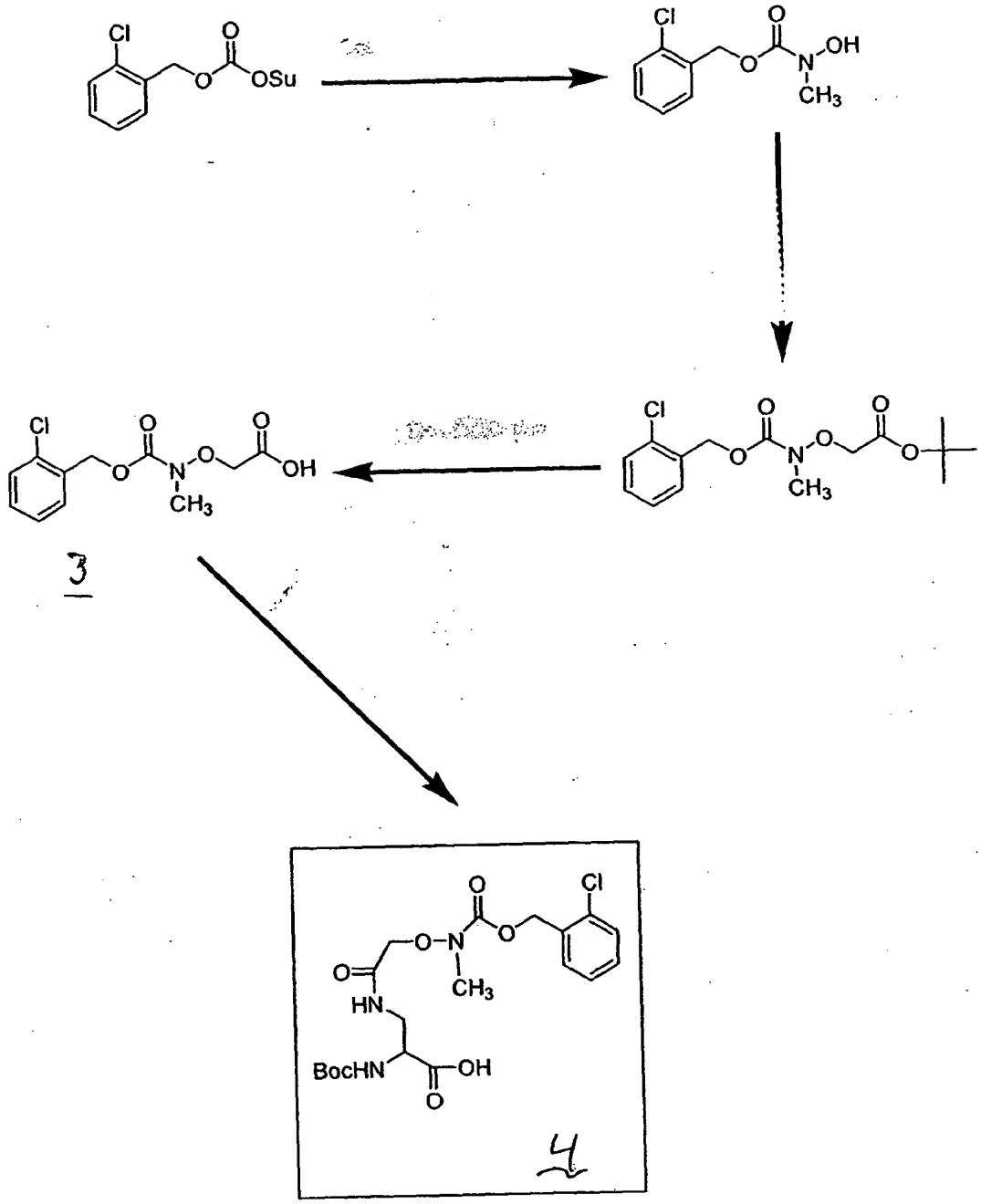

Cl
O
OSu
Cl
O
OH
N
CH3
Cl
O
O
N
O
OH
CH3
Cl
O
O
N
O
O
O
CH3
Cl
O
O
N
O
O
OH
CH3
3
O
Cl
O-N
O
O
CH3
HN
BocHN
OH
O
4

Figure 5

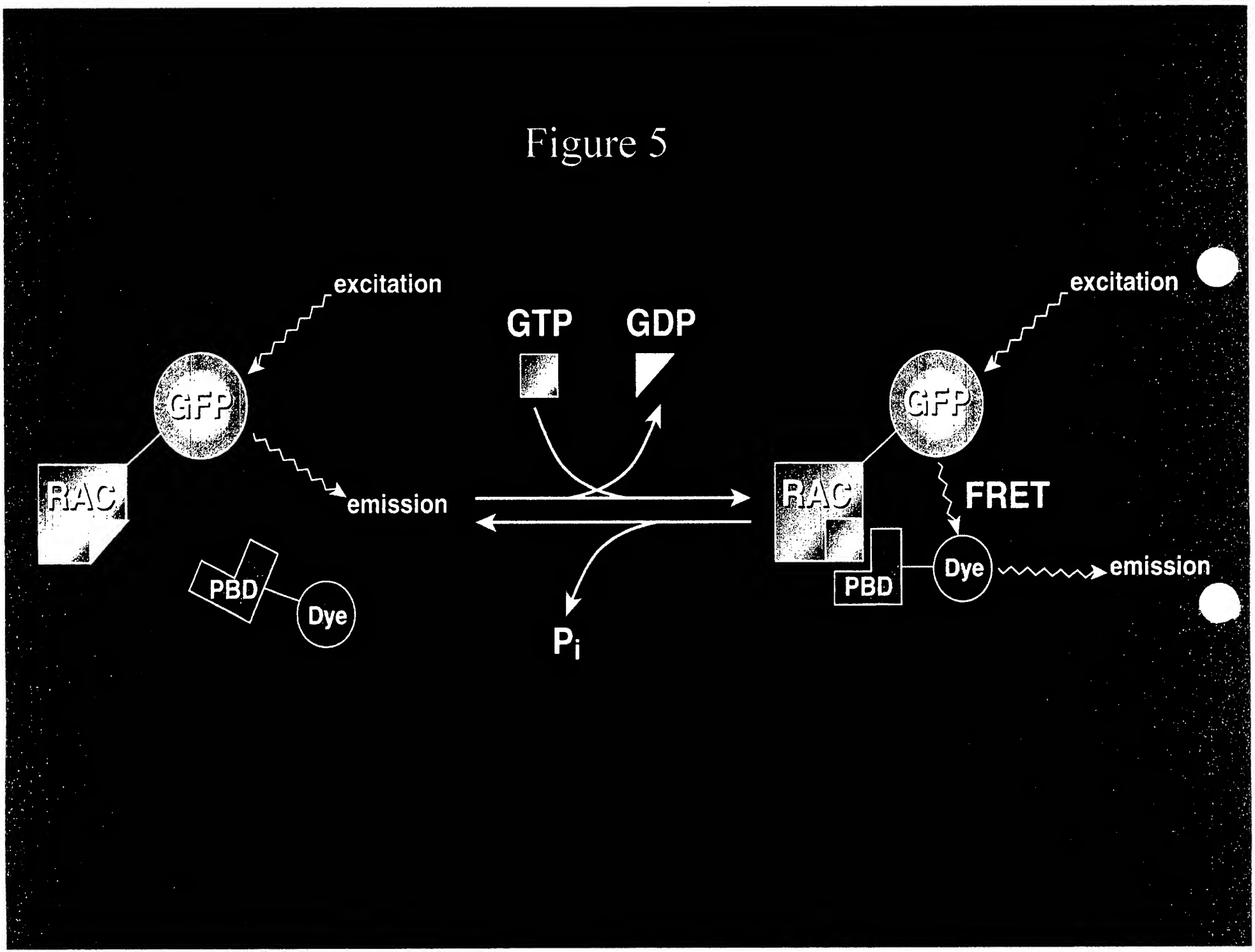
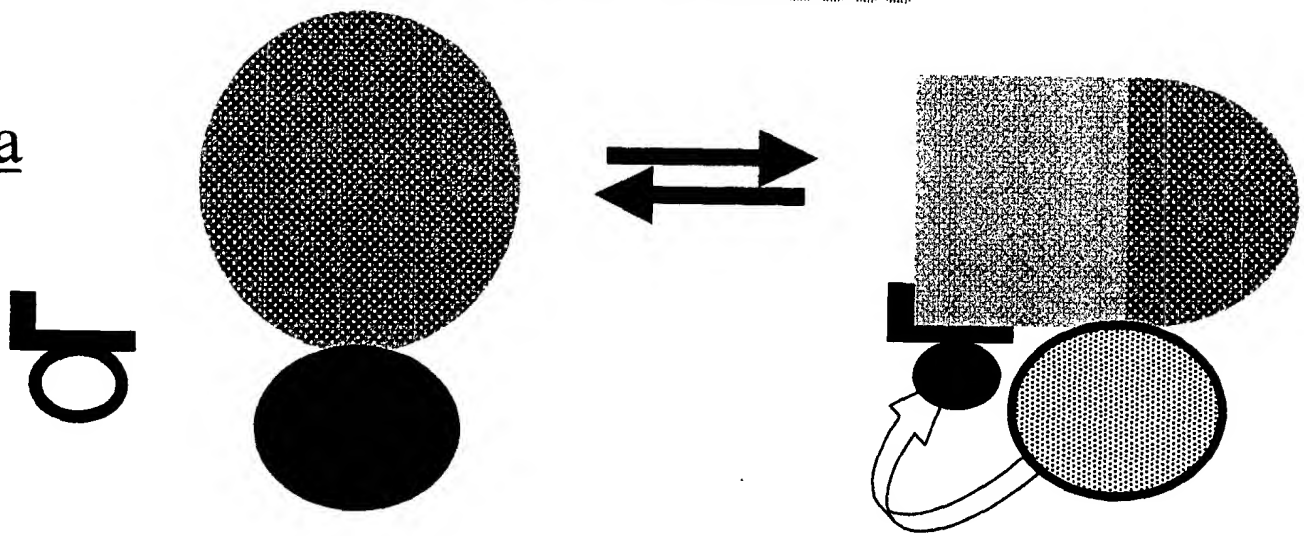

excitation
GFP
RAC
emission
PBD
Dye
GTP
GDP
Pi
excitation
GFP
RAC
FRET
PBD
Dye
emission

Fig. 6a



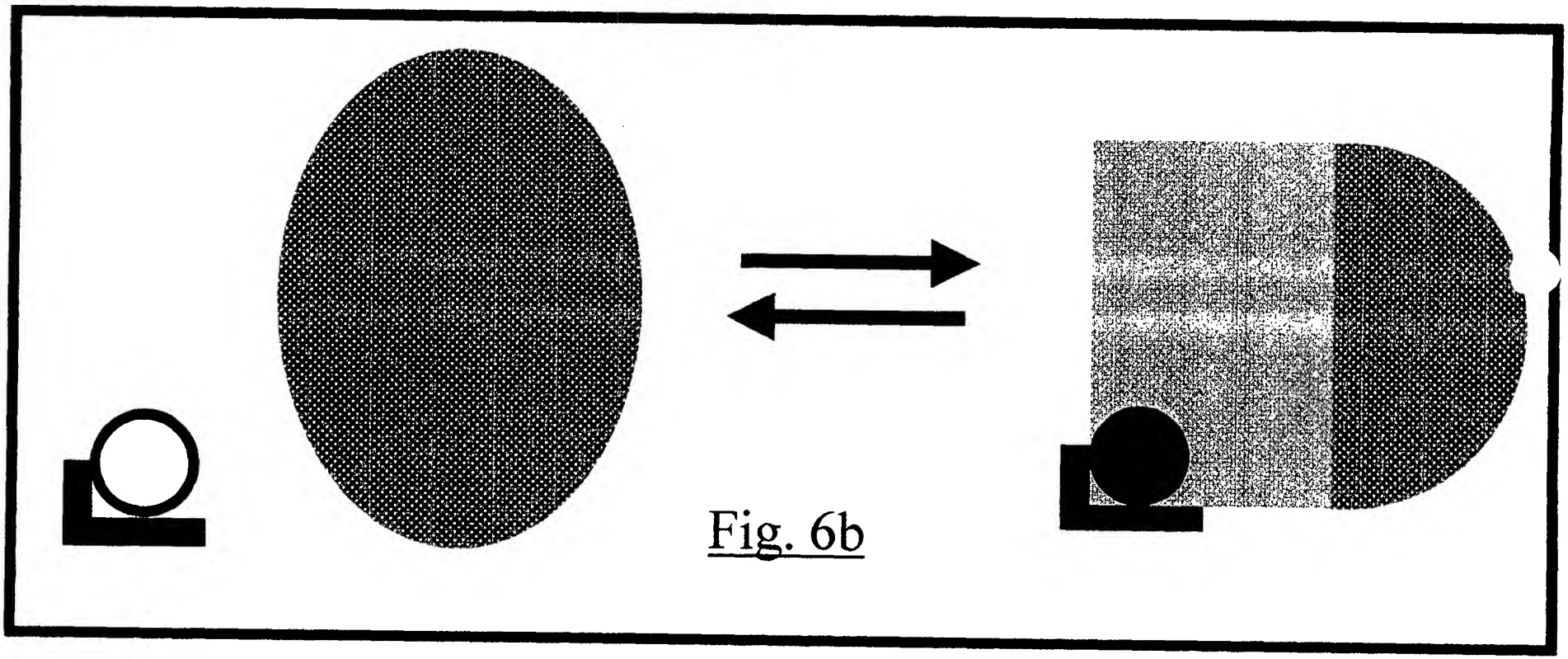


Fig. 6b

Figure 7A: GFP-Rac to Alexa-PBD FRET

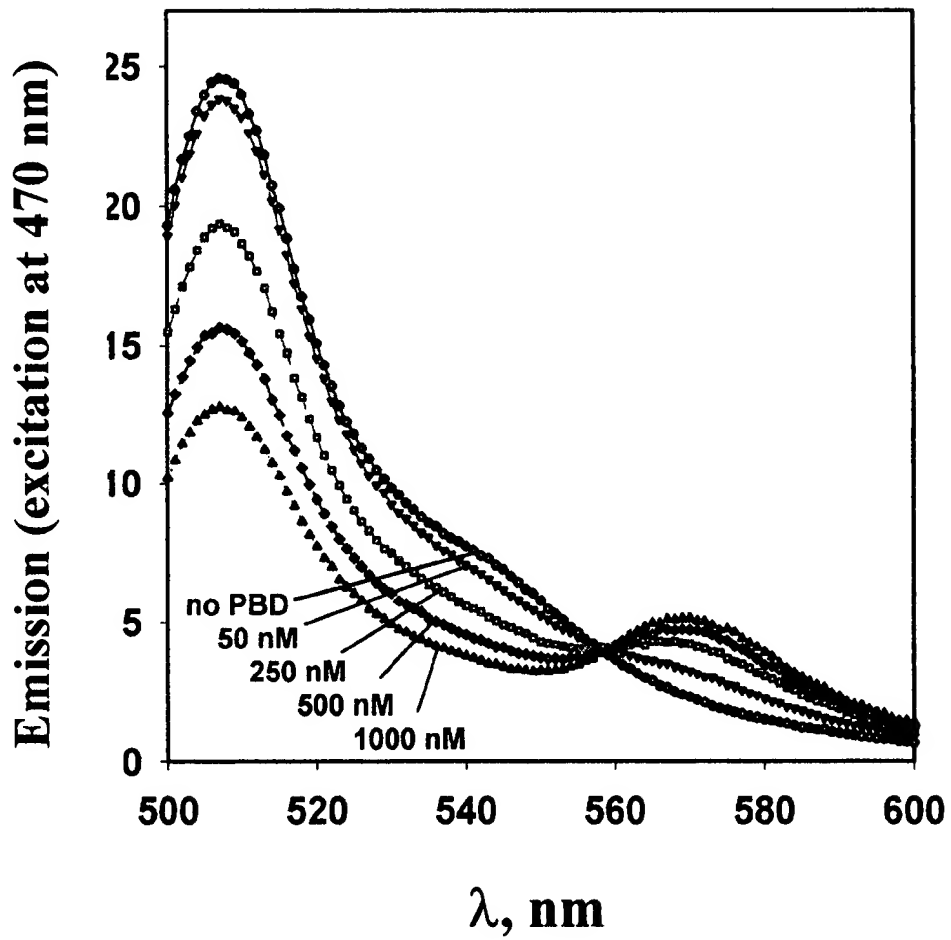


Figure 7B: FRET response to nucleotide state of Rac-GFP

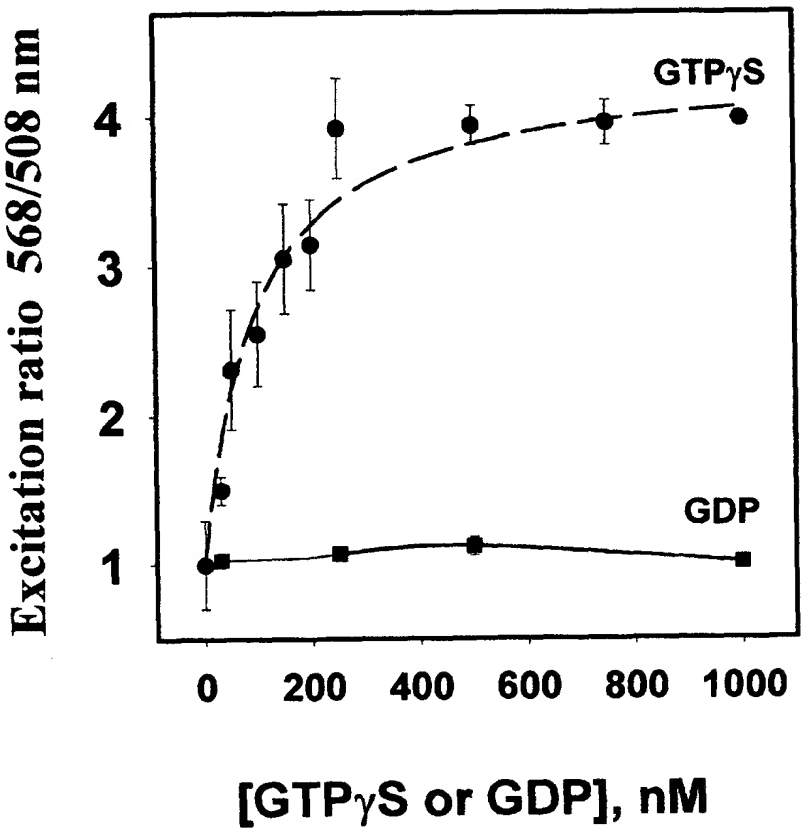
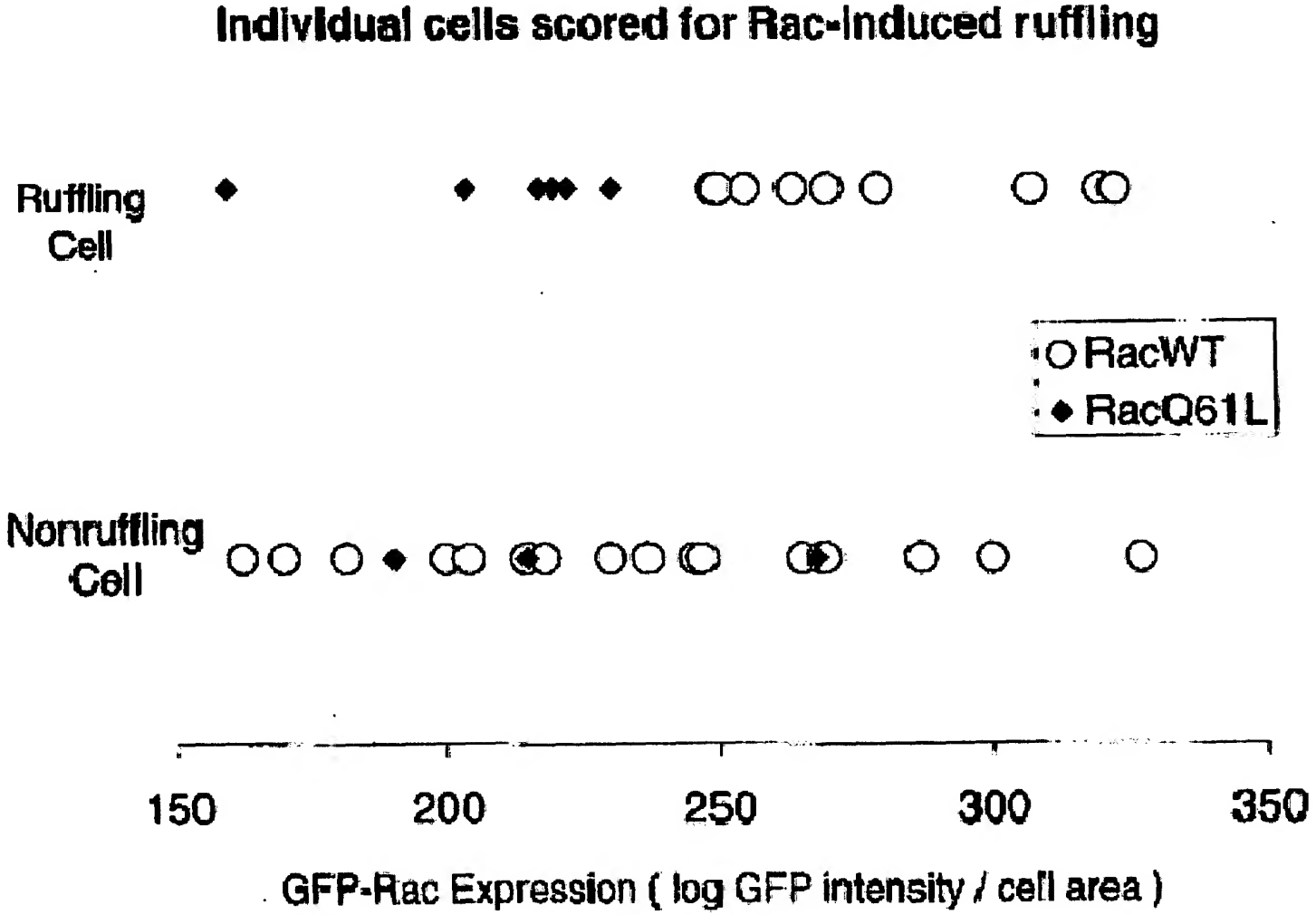


Fig. 8A


Individual cells scored for Rac-induced ruffling
Ruffling Cell
Nonruffling Cell
RacWT
RacQ61L
150
200
250
300
350
GFP-Rac Expression (log GFP intensity / cell area)

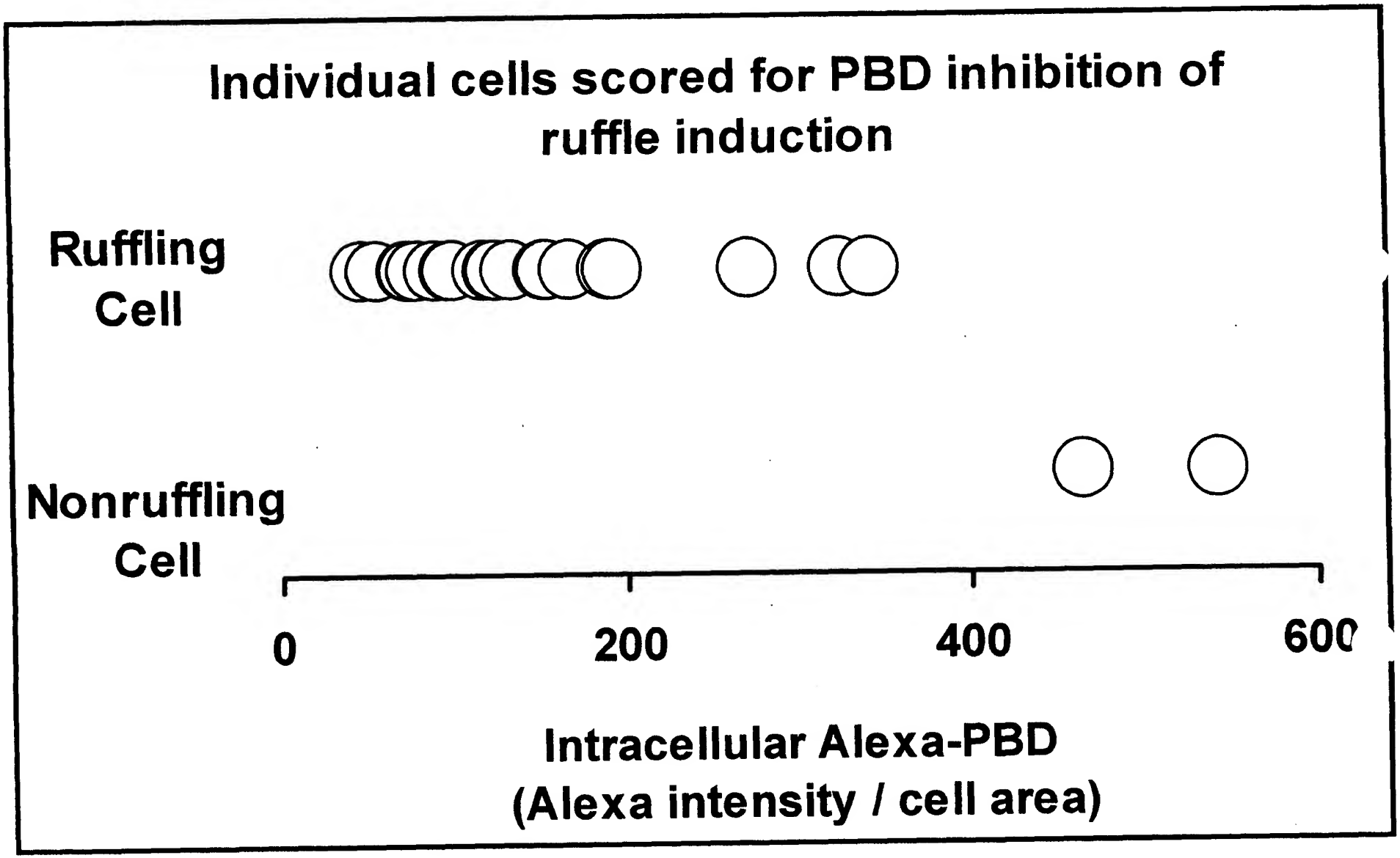

Individual cells scored for PBD inhibition of ruffle induction
Ruffling Cell
Nonruffling Cell
0
200
400
600
Intracellular Alexa-PBD
(Alexa intensity / cell area)

Figure 8B

Fig. 9 A and 9B: Serum stimulation of a Swiss 3T3 fibroblast

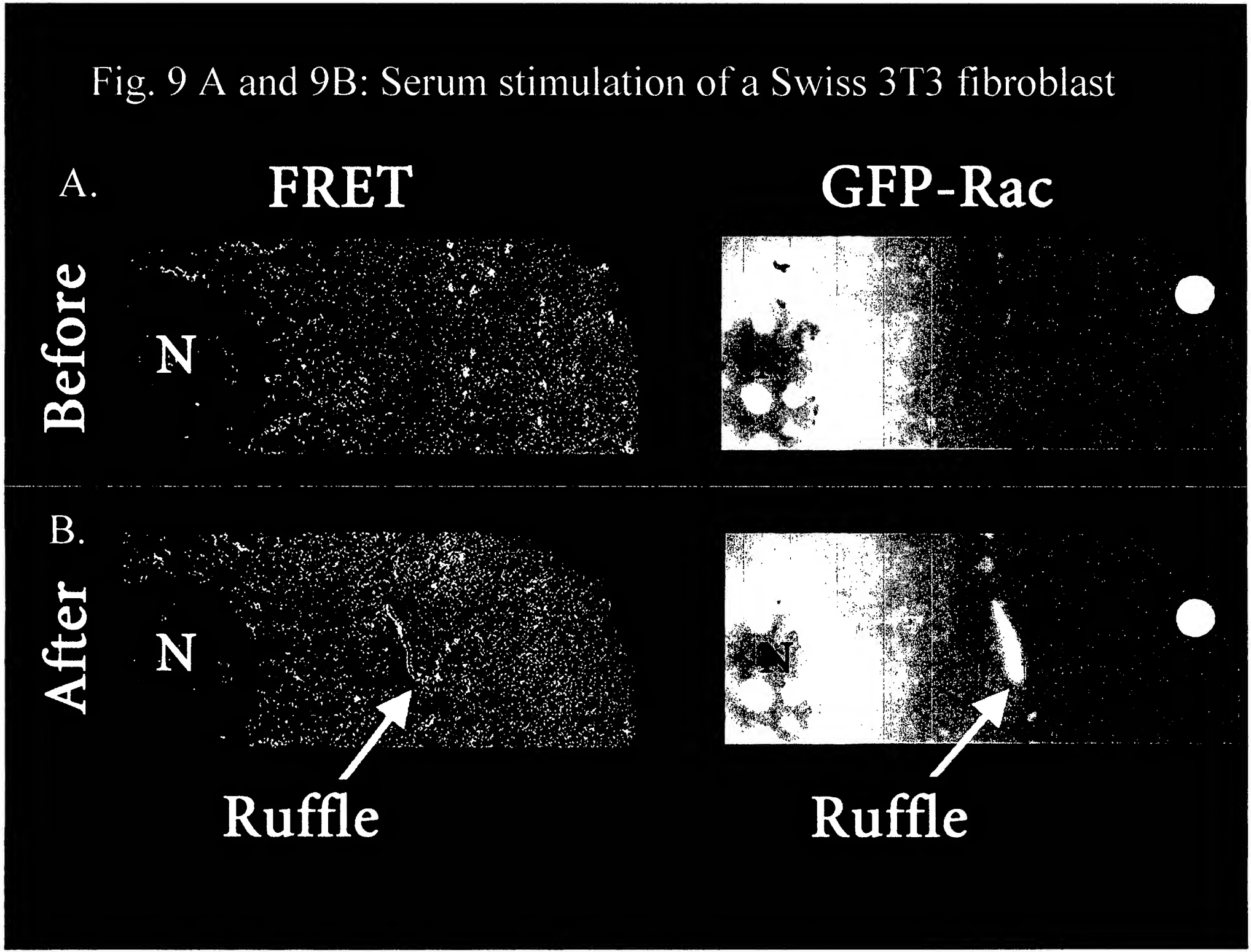


Fig. 9C and 9D: The same ruffle visualized using either FRET or Alexa-PBD localization:

C. FRET
intensity = 0-84

D. Alexa-PBD
intensity = 88-345

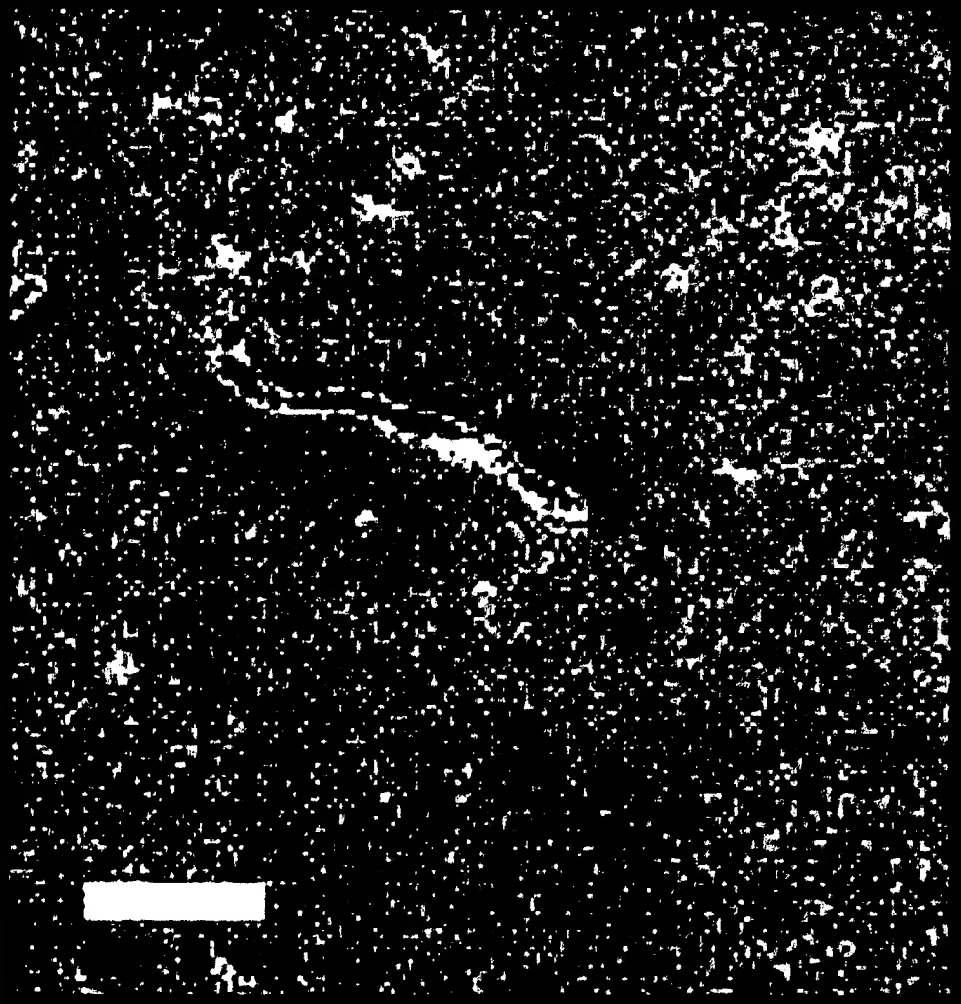


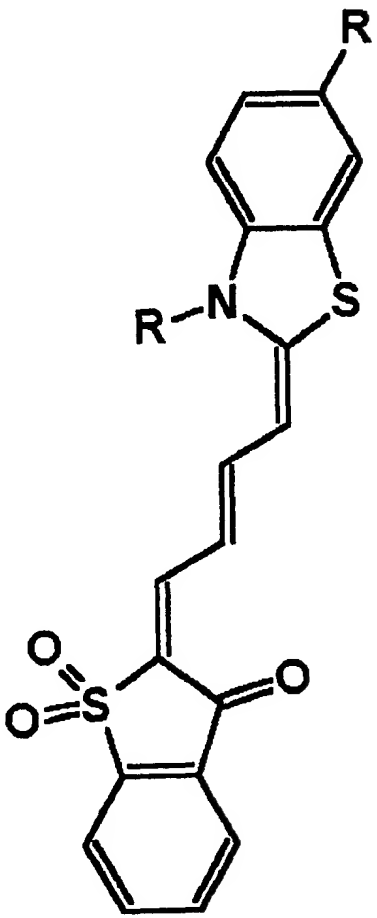
Fig. 10A: Rac-GFP

Fig. 10B: FRET

Wound healing

Confluent monolayer

Magnitude of gradient when highest at front	128 +/- 51 %	n=12
Magnitude of gradient highest at rear	9 +/-4 %	n=4


R
R
N
S
O
O
S
O

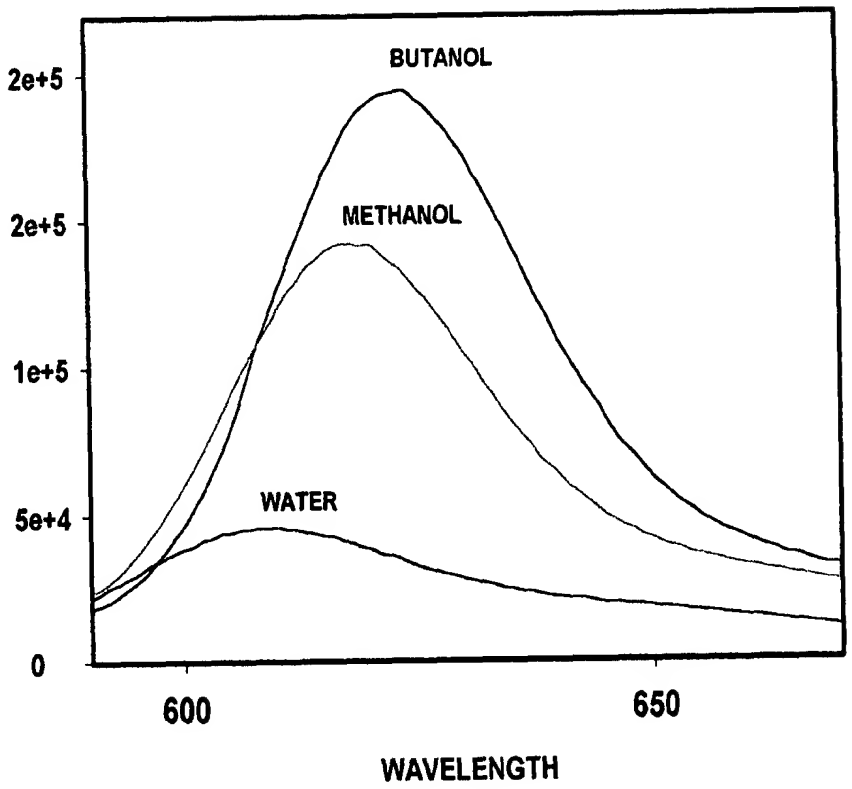
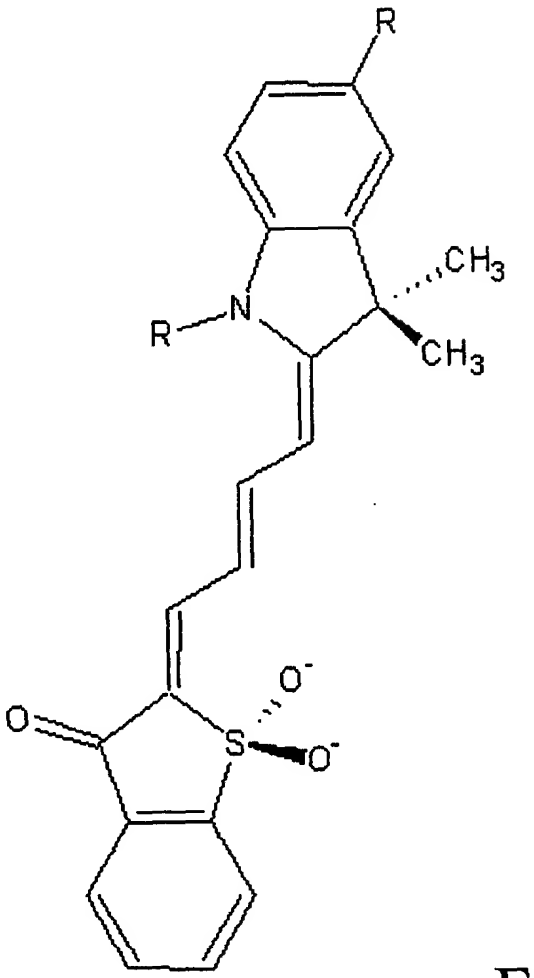

BUTANOL
METHANOL
WATER
2e+5
2e+5
1e+5
5e+4
0
600
650
WAVELENGTH

Figure 11


R
CH3
N
R
CH3
O
O-
S
O-

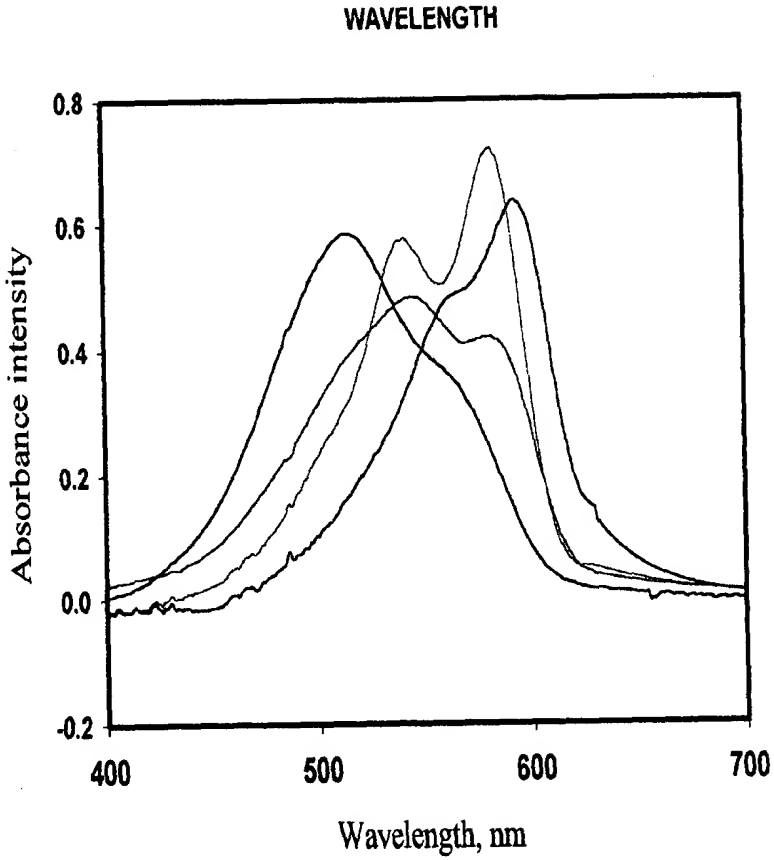
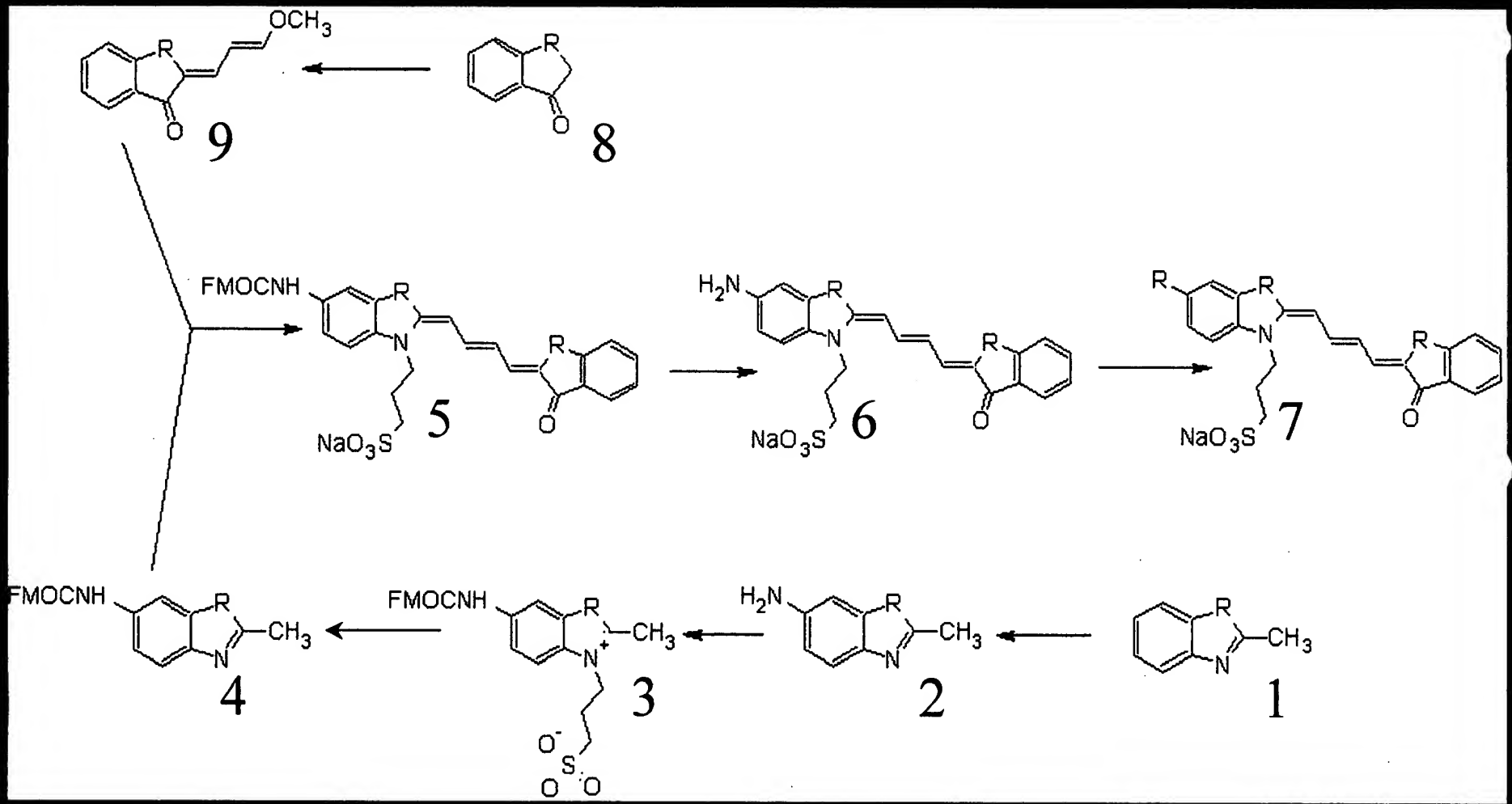

WAVELENGTH
Absorbance intensity
0.8
0.6
0.4
0.2
0.0
-0.2
400
500
600
700
Wavelength, nm

Figure 12

Figure 13: Convergent synthesis of merocyanine dyes



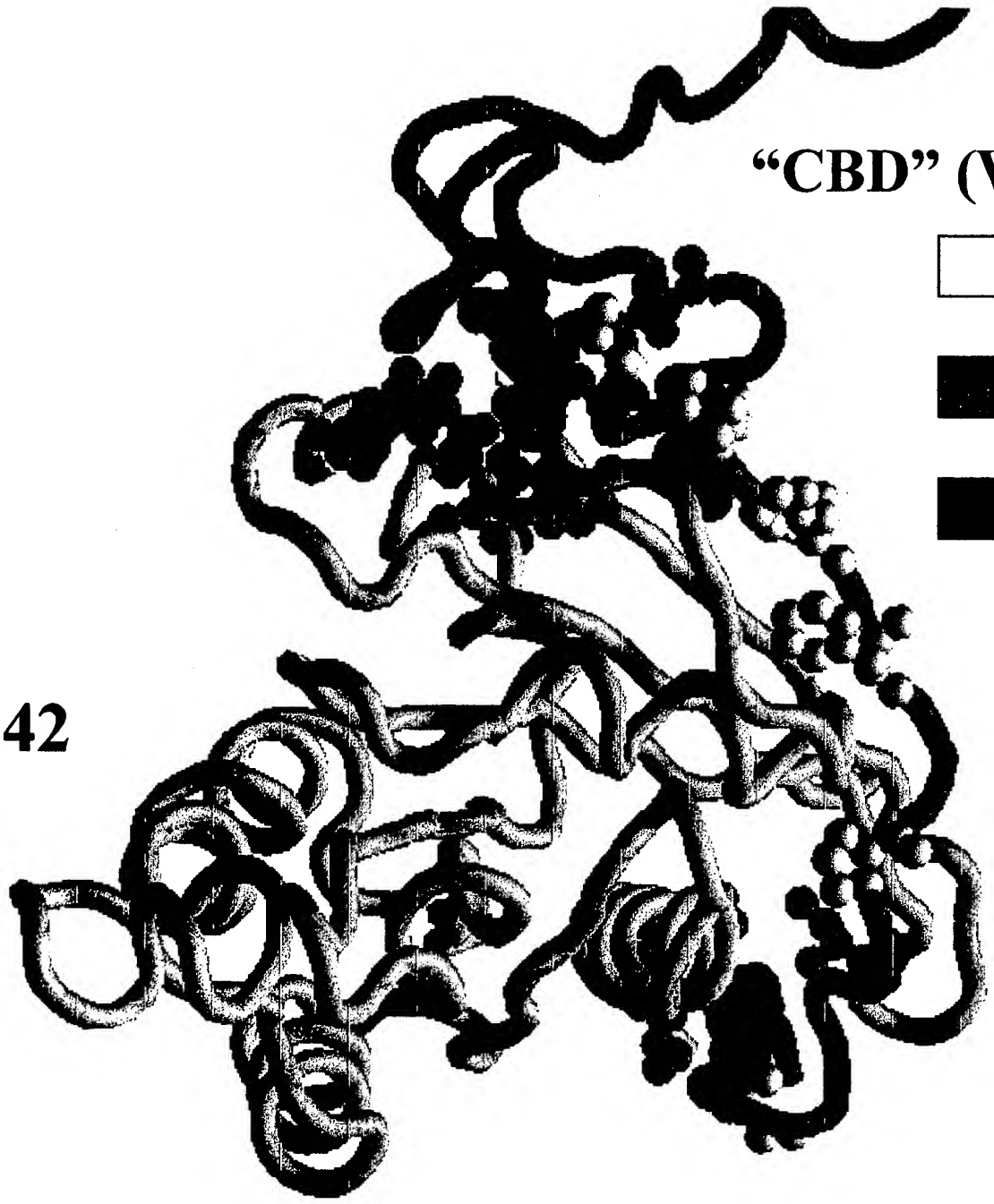


Figure 14:

"CBD" (WASP CRIB domain)

essential residues

sites of dye attachment

hydrophobic amino acids

Fig. 15: Fluorescence of Mero-CBD responds to Cdc42 binding

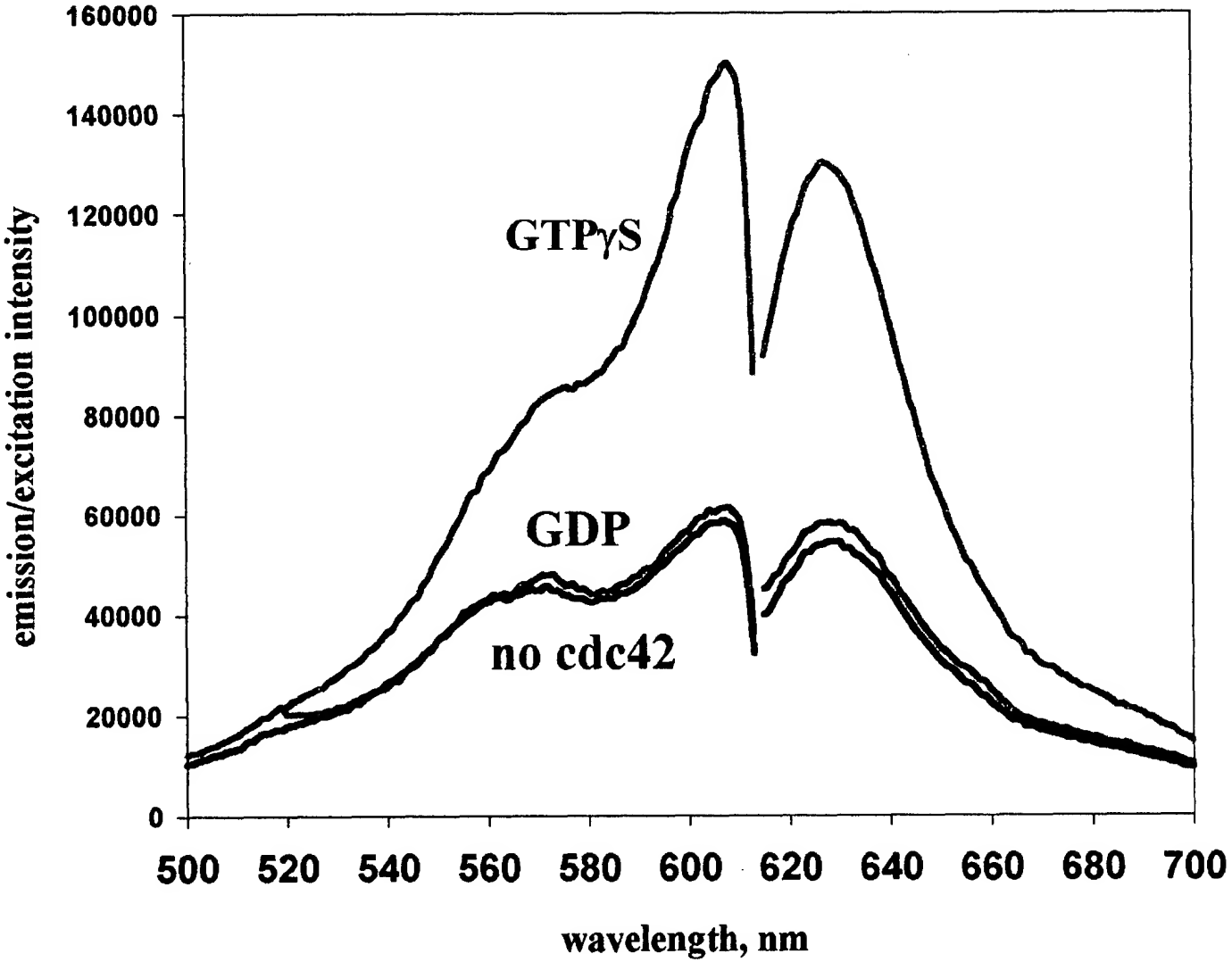


Fig. 16: **Mero-CBD in neutrophil lysates**

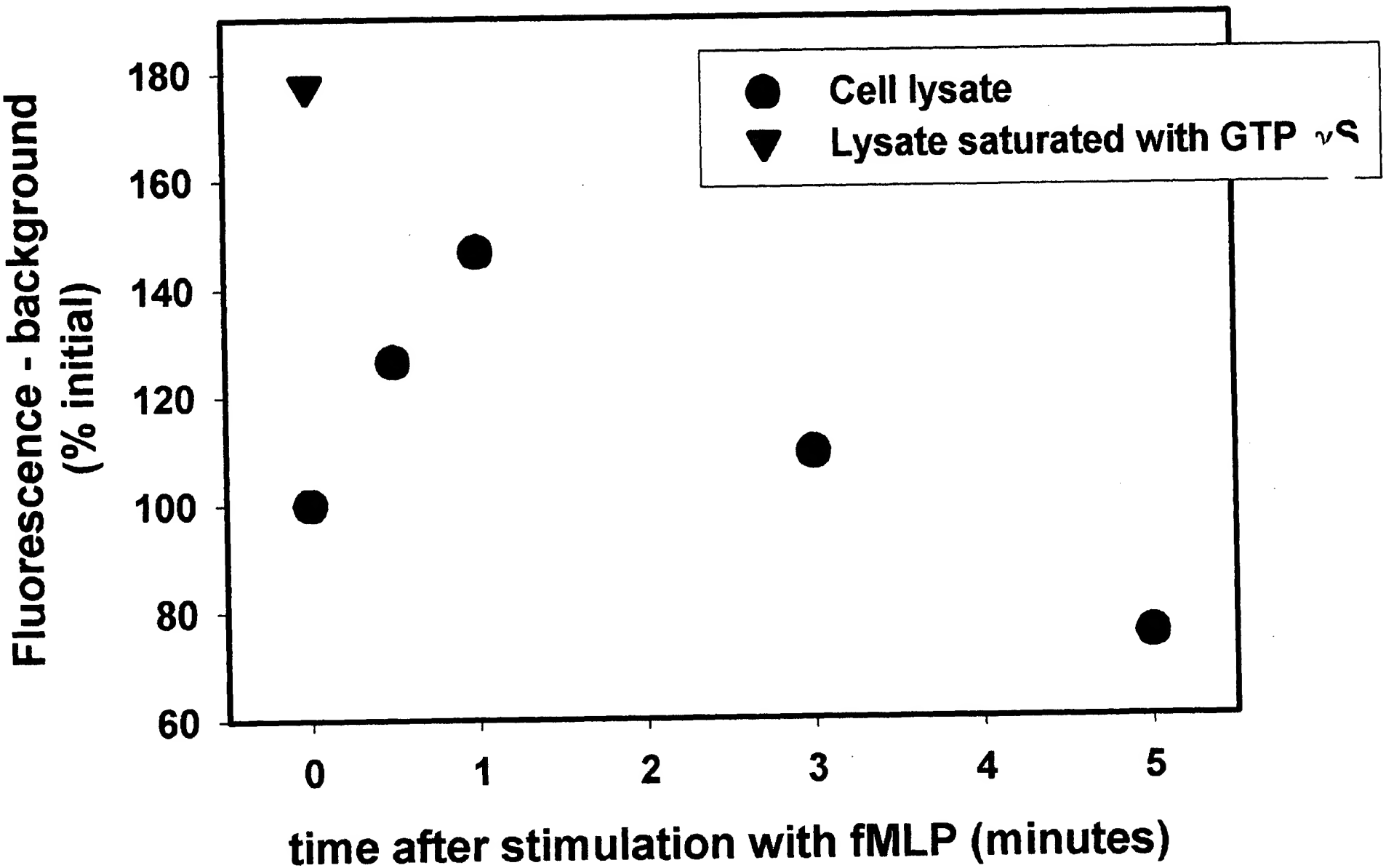


Fig. 17